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Aliphatic and Aromatic Biomarkers for Petroleum Hydrocarbon Investigation in Marine Sediment

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Abstract

Levels, composition profiles and sources of hydrocarbons were analyzed in surface marine sediment samples collected from Khniss Coast in Tunisia. It was found that the total Hydrocarbon (TH) concentrations ranged from 2280 μg/g to 7700 μg/g. The sedimentary non-aromatic hydrocarbon (NAH) and aromatic hydrocarbon (AH) concentrations ranged from 1020 to 2320 μg/g, and from 240 to 680 μg/g, respectively. The level of 17 polycyclic aromatic hydrocarbons (∑17PAHs) is equal to 14.59 ng/g. The PAH profiles showed that the ∑4–5-ring compounds were the major PAHs detected in the sampling sites. Characteristic ratios of Anth/(Anth+ Phe), and Flu/(Flu + Pyr) indicated that PAHs could originate from petrogenic and pyrolytic sources. Petroleum contamination associated with increased marine activity and high eutrophization statue in Khniss area which may have side-effects on the ecosystems and human safety; thus, it must be controlled.

Keywords

Marine Sediments; Petroleum Pollution; Aliphatics; Aromatics; PAH; Biomarkers

Introduction

Petroleum pollution has become a matter of serious environmental concern all over the world, because of its extensive use as energy source which has led to its widespread distribution in the biosphere (Diya'uddeen et al., 2011). Petroleum hydrocarbons (PHCs) come into the environment through accidents, spills or leaks, from industrial releases, or by products from commercial or domestic uses (Ou et al., 2004; Mille et al., 2007). PHCs are complex mixtures in both composition and molecular structure, mostly originating from crude oil. PHCs contain a wide range of chemical products such as gasoline, kerosene, fuel oil, heavy oil and lubrication oil. The total input of petroleum into the oceans through human activities, atmospheric fallout and natural seepage is estimated at 2.37 x 106 tones per year. Out of these, about 65% is discharged through household and industrial wastes, urban and river runoffs, oceanic dumping and atmospheric fallout; 26% is derived from discharge during transportation, dry docking, tanker accidents, and de-blasting (GESAMP, 1993). The fate of petroleum hydrocarbons in marine environment is subject to complex and interrelated physic-chemical processes that include evaporation, dissolution, photo-oxidation, microbial degradation, emulsification and sedimentation. These physical and chemical factors cause important modifications to the hydrocarbon compounds, which render them difficult to detect and to analyze (Wang and Fingas, 2005; Venkatachalapathy et al., 2011). The contamination of marine sediment by PHCs is widespread in coastal regions and represents a major concern for the potential detrimental consequences on ecosystems' health and provision. PHCs in water and air may reach the sediment by adsorption and deposition. Sediment becomes a primal carrier and environmental termination of PHCs (Michael et al., 2002). Sediment system can be considered as a major reservoir and sinks for marine pollutants because of its holding capacity for organic pollutants (Mille et al., 2007; Choi et al., 2011). It can also be a good indicator of environmental pollution (Baird et al., 2005;

Baudouin et al., 2007). Analyzing the composition of hydrocarbon compounds in marine sediments can provide much information about their sources and digenetic processes and reflect the extent of anthropogenic pressures on the environment (Hostettler et al., 1999; Medeiros et al., 2005; Zaghden et al., 2005). Therefore, non-aromatic hydrocarbons (NAHs), such as n-alkanes, unresolved complex mixture (UCM), and polycyclic aromatic hydrocarbons (PAHs) measured in sediment have been used to assess petroleum contamination in the marine environment (Beg et al., 2003; Ye et al., 2007; Da Silva et al., 2010; Maioli et al., 2010). The sources of these environmental geochemical markers are both natural and anthropogenic (Budzinski et al., 1997). Recent studies have examined the hydrocarbon status in Tunisian marine coasts (Louati et al., 2001; Zaghden et al., 2005; 2007; Trabelsi et al., 2005; Khedir-Ghenim et al., 2009) but to our knowledge, no information on hydrocarbon levels and origins in Khniss region is available. The Khniss area is situated at the Middle Eastern part of Tunisia. This coastal area is subject to important fishing activities and it receives several domestic wastes from the surrounding areas and especially the discharge of water from Wadi Khniss. Therefore, the identification and quantification of PHC compounds in this marine area will be of the utmost interest. The purposes of the present study were: i) to evaluate the hydrocarbons contamination levels from superficial marine sediment in the Khniss coastal area of Tunisia, ii) to obtain detailed information on the spatial distribution of the different fractions AH, NAH and PAH, iii) to determine the eventual sources for each fraction by using geochemical biomarkers.

Materials and Methods

Area of Study and Sampling

The area under study was located between 35°42′08.77′′N; 10°49′54.42′′E and 35°43′06.32′′N, 10°49′02.76′′E of Tunisia using the Global Positioning System (GPS). Sample collection occurred in 2008. Sediment samples were collected from superficial layer at four different sampling sites identified as Keniss Sediment-A (KSA), Keniss Sediment-B (KSB), Keniss Sediment-C (KSC) and Keniss Sediment-D (KSD). The coordinates of sampling sites are given in Table 1. The sediment samples were stored in pre-cleaned aluminum boxes at 4°C during transportation to the laboratory (with no exposure to light). The characteristics of the sediment samples were described in Table (1). At each site, three samples, separated by 3 m from each other, were taken. In our laboratory, the three sediment samples of the same site were mixed in order to increase the size of the samples and to recover the surface of sampling, manually homogenized, lyophilized, passed through a stainless steel sieve (200 and 100 μm) and finally stored at 4◦C until analysis. All the results were reported as dry weight.

Hydrocarbons Analysis

The total hydrocarbons (TH) present in 20 g of dry weight (dw) sediment were extracted in a Soxhlet apparatus with chloroform for 16 h. Recovery ranged from 97.4% to 98.9% for the n-eicosene. Following chloroform evaporation, the extract was fractionated into aliphatic and aromatic hydrocarbons by adsorption liquid chromatography using a column of alumina and silica-gel, and gradient solvents as eluent: n-hexane and 2:1 n-hexane/ chloroform for non-aromatic hydrocarbon (NAH) and aromatic hydrocarbon (AH) fractions, respectively. The NSO fraction (PF) was not eluted and calculated according to: NSO= TH – (NAH +AH). The total petroleum hydrocarbon (TPH) represents the sum of the NAH and AH fractions. Fractionation was performed in a silica micro-column (silica gel 60 of 63–200 μm). Prior to use, the silica was cleaned with chloroform, subjected to a one-hour wash at 50°C under magnetic agitation, and filtered through a glass fiber filter (type GF/A, 47 mm diam., 1.6 μm retention) (Whatman International, Kent, UK). The silica was then conditioned overnight at 110°C. Following solvent evaporation, the NAH and AH were weighed. The NAH were analyzed with a Hewlett-Packard 5890 gas chromatograph equipped with a temperature-controlled injector, a flame ionization detector (GC/ FID), and a capillary column HP5: 5%diphenyl, 95% dimethylpolisiloxane (25 m x 0.32 mm x 90.52 μm). The oven temperature program was as follow: 1 min at 80°C, from 80 to 280°C at 4°C/min and 10 min at 280°C. The injector and detector temperatures were 250°C and 280°C, respectively. The samples were solubilized in cyclohexane and 1 μl of this sample was injected following the addition of external standard (*n*-eicosene).

Aliphatic Hydrocarbon Analysis

Quantification of the total resolved (TR) hydrocarbon (n-alkanes) and unresolved complex mixtures (UCM) were calculated using the mean response factors of n-alkanes. Each hydrocarbon concentration was expressed as dry weight (dw). Recovery assays ranged from 94.50% to 98.12% for C18 nalkane and from 87.0% to 92.3% for a mixture of C10–C34 n-alkanes. Concentrations of individual n-alkanes (n-C12 to n-

C32), isoprenoids pristane and phytane, TR, UCM, and total aliphatic (TA) fraction (sum of identifiable aliphatic peaks + UCM) were calculated. The detection limit was determined at 0.001 μg/g for the C18 n-alkanes. Data of the TH, NAH, and AH concentrations are given as means ± SD (n = 3).

Aromatic Hydrocarbon Analysis

The AH were analyzed with a Hewlett-Packard 5890 gas chromatograph equipped with a temperature-controlled injector, a flame ionization detector (GC/FID), and a capillary column HP5: 5%diphenyl, 95%dimethylpolisiloxane (25 m x 0.32 mm x 90.52 μm) under the same temperature program and the same analytic conditions described for the NAH fraction (see section 1.2). The Gas Chromatography (GC) identification and quantification of PAHs was carried out as described by (Trablsi et al., 2005) based on the comparison with known standards injected under the same conditions. A certified standard reference (National Institute of Standards and Technology, USA) was used. Sixteen un-substituted PAHs have been listed by the US Environmental Protection Agency (EPA) as priority pollutants. The PAHs investigated in this study were: naphthalene (Naph), 1-methyl-naphthalene (1menaph), 1-ethyl-naphthalene (1enaph), ace-naphthylene (Ac), acenaphthene (Ace), 2,3,6-trimethyl-naphthalene (2,3,6- trimenaph), fluorene (Flu), phenanthrene (Phe), 2-methyl-phenanthrene (2-mephe), 1- methyl-phenanthrene (1-mephe), 3,6-dimethyl-phenanthrene (3,6-dimephe), fluoranthene (Fluo), pyrene (Pyr), 1-methyl-pyrene (1-mepyr), anthracene (Anth), chrysene (Chr), and perylene (Pery). When the peaks were not identified by GC, an analysis was carried out using a coupled GC/MS. The mass spectrometer was of type HP 9572 II (Agilent, California) (GC/MS) equipped with a Splitless injection system. The capillary column (30 m, 0.25 mm, 0.25 μm), the carrier gas was nitrogen. The GC conditions were the same as described (1.2 Hydrocarbon analysis) for CG/FID analysis. For the MS analysis, the electron ionization of 70 eV and linear scanning over the mass range 35–500 Da were used. Compound identification was based on individual mass spectra and GC retention times in comparison to the literature, library data, and standards. To ensure an appropriate quality of analyses, standards and blanks were analyzed under the same conditions as the samples. All analyses were done in triplicate.

Statistical Analyses

Data of the different fraction of hydrocarbon concentrations were statistically analyzed. For each fraction, standard errors were calculated between three repetitions analysis (n = 3). Results are expressed as mean SE (standard error). Comparisons among multiple groups of samples, for each site, were achieved by one-way ANOVA followed by Tukey's Multiple Comparison Test. Statistical significance was defined as $P < 0.05$.

TABLE 1 LOCATIONS AND CHARACTERISTICS OF SAMPLING SITES IN KHNISS COAST FROM TUNISIA

Site	Station code	Localization		Size of sediment	Type of bottom	Depth
KSA	KSA-1	35°43′06,32′′N	10°49′02,76′′E	Fine grained sand<100 μm	Sand without seagrass	0-15 cm
	KSA-2	35°43′02,79′′N	10°49′12,46′′E	Fine grained sand<100 μm	Sand without seagrass	0-15 cm
	KSA-3	35°43′01,18′′N	10°49′05,71′′E	Fine grained sand<100 μm	Sand without seagrass	0-15 cm
KSB	KSB-1	35°42′52,51′′N	10°49′18,36′′E	Fine grained sand<100 μm	Meadow of seagrasses	0-15 cm
	KSB-2	35°42′47,37′′N	10°49′21,32′′E	Fine grained sand<100 μm	Meadow of seagrasses	0-15 cm
	KSB-3	35°42′44,60′′N	10°49′23,02′′E	Fine grained sand<100 μm	Sand without seagrass	0-15 cm
KSC	KSC-1	35°42′34,87′′N	10°49′25,64′′E	Fine grained sand<100 μm	Sand without seagrass	0-15 cm
	KSC-2	35°42′31,45′′N	10°49′32,98′′E	Fine grained sand<100 μm	Sand without seagrass	0-15 cm
	KSC-3	35°42′28,06′′N	10°49′31,32′′E	Fine grained sand<100 μm	Sand without seagrass	0-15 cm
KSD	KSD-1	35°42′14,68′′N	10°49′37,95′′E	Fine grained sand<100 μm	Meadow of seagrasses	0-15 cm
	KSD-2	35°42′05,38′′N	10°49′47,71′′E	Fine grained sand<100 μm	Meadow of seagrasses	0-15 cm
	KSD-3	35°42′08,77′′N	10°49′54,42′′E	Fine grained sand<100 μm	Meadow of seagrasses	0-15 cm

KSA, KSB, KSC and KSD : sampling site.

TABLE 2 HYDROCARBON CONCENTRATIONS (μG/G ± SE DRY WEIGHT) IN MARINE SEDIMENTS FROM KHNISS COASTAL

Site reference	TH	NAH	%NAH	AH	%AH	NSO	%NSO	TPH	%TPH	TPH/NSO
KSA	4060±24.2	2260±20.3	55.6	240±9.1	5.9	1560±15.7	38.5	2500±11.9	61.5	1.6
KSB	7700±48.4	1650±12.8	21.2	340±11.4	4.4	5710±26.2	74.3	1990±10.8	25.7	0.34
KSC	2280±17.1	1020±14.2	44.7	330±8.4	14	930±19.7	41.3	1350±16.4	58.7	1.45
KSD	7050±21.1	2320±16.1	32.9	680±14.2	9.6	4050±21.4	57.4	3000±23.2	42.6	0.74

KSA, KSB, KSC and KSD: sampling site.

TH: Total hydrocarbons; NAH: Non aromatic hydrocarbons; AH: Aromatic hydrocarbons; NSO: Heavy compounds; TPH: Total petroleum hydrocarbons. These are expressed as μg/g dwt of sediment by using gravinemetry analyses.

Results and Discussion

Distribution of TH, NAH and AH in Sediment

The sediment samples from KSA, KSB, KSC and KSD were subjected to an organic analysis to determine the biogenic and anthropogenic hydrocarbon input in the region. Results of total hydrocarbons, non-aromatic hydrocarbon, aromatic hydrocarbon and total petroleum hydrocarbon were reported in Table 2. Results show high TH levels in all the sites of study, which may be due to anthropogenic, terrestrial and marine organic matter inputs. TH concentrations differed from one site to another. KSB and KSD registered the highest TH levels with 7700 ± 48.4 μg/g dw and 7050 ± 21.1 μg/g dw, respectively. These high levels could be due to the contribution of biogenic hydrocarbons in these sites as confirmed by the significant dominance of heavy fraction (NSO) in both KSB and KSD. Similar results were reported in the northern coast of Sfax-Tunisia (1127 μg/g and 5217 μg/g) (Louati et al., 2001). Total hydrocarbon levels in the Jarzouna Mediterranean coastal area ranged from 602 ± 7.638 μg/g dw to 1270 ± 2.176 lg/g dw (Khedir-Ghenim et al., 2009). These levels were higher than those found in the Gulf of Fos area of France (10.3–260.0 μg/g) (Mille et al., 2007). Biogenic hydrocarbons are generated either by biological processes or in the early stages of digenesis in recent marine sediments. Biological sources include land plants, phytoplankton, animals, bacteria, macroalgae, and microalgae (Wang et al., 2003). Furthermore, NAH and AH levels showed variations among the collection sites, with an average of 1020±14.2-2320±16.1 μg/g for NAH and 240±9.1-680±14.2 μg/g for AH (Table 2).

The highest NAH levels were found in KSA and KSD sampling sites, while the most elevated aromatic fraction level is associated to KSD site. NAH are predominant in the TPH fraction in each sediment sample. NAH percentages varied from 21.2% to 55.6%, while the aromatic percentages varied from 4.4% to 14 %. These percentages are comparable to those found in previous study (Zrafi-Nouira et al., 2008) in the Jarzouna region in Bizerte (Tunisia) confirming petroleum origin of hydrocarbons. The ratio of TPH fraction/heavy hydrocarbons (NSO) may indicate the origin of organic matter in sediment. KSB and KSD sites have a ratio TPH/NSO <1, which indicates biogenic origin of hydrocarbons (Table 3). On the other side, KSA and KSC have a ratio TPH/NSO >1, which confirms petroleum origin of hydrocarbons from these sites. KSA and KSC sediment hydrocarbons displayed typical characteristics of crude oil, as the TPHs were over 50%. Our results showed biogenic input in the KSB and KSD sites, and suggest petroleum origin in sediments from KSA and KSC. We also demonstrated that the total hydrocarbon levels in the Khniss Mediterranean coastal area ranged from 2280±17.1 μg/g dw to 7700±48.4 μg/g dw (Table 2). These levels were higher than those found in the northern coast of Sfax-Tunisia (1127-5217 μg/g) (Louati et al., 2001). Total hydrocarbon concentrations can be classified as relatively high by comparison with other coastal Mediterranean sediments (Grimlat et al., 1990; Le Dreau et al., 1981; Mille et al., 2007) and other coastal sediments (Massoud et al., 1998; Beg et al., 2003, Gao et al., 2008). The NAH levels recorded in all the sites are relatively high (Table 3) compared to those recorded in the area of Sfax (Elloumi et al., 2008), which vary from 26.7 to 127.8 μg/g and those recorded (Commendatore et al., 2004) in the Patagonia region in Argentina, ranging from 0.27 to 1304.7 μg/g. Levels of

TABLE 3 LEVELS OF NAH (μg/g DRY WEIGHT) IN THE TWO POLLUTED SITES (KSA AND KSC) FROM SEDIMENT OF KHNISS

	KSA (μg/g)	**KSC (μg/g)**
C9	4 .55 10^{-01}	4.55 10^{-01}
C10	1.15	1.15
C12	2.24 10^{-03}	2.24 10^{-03}
C13	8.94 10^{-05}	8.94 10^{-05}
C14	2.8 10^{-03}	2.80 10^{-03}
C15	2.99 10^{-03}	2.99 10^{-03}
C16	3.47 10^{-02}	3.47 10^{-02}
Pr	3.24 10^{-01}	3.24 10^{-01}
C17	1.18 10^{-01}	1.18 10^{-01}
Ph	3.00 10^{-01}	3.00 10^{-01}
C18	9.50 10^{-01}	9.50 10^{-01}
C19	1.37	1.37
C20	2.15	3.03 10^{-01}
C21	1.43	2.10 10^{-01}
C22	1.70	1.2
C23	1.18	2.63 10^{-01}
C24	1.99	4.21 10^{-01}
C25	2.96	2.49 10^{-01}
C26	4.36	2.91 10^{-01}
C27	4.79	4.63 10^{-01}
C28	3.9	5.20 10^{-02}
C29	3.39	7.41 10^{-02}
C30	2.09	7.59 10^{-02}
C31	1.37	1.37
C32	ND	ND
Σn-alcanes	36.08	9.69
UCM	142.12	65.43
ΣLMW	6.88	5.02
ΣHMW	29.2	4.67

KSA and KSD: sampling site. C1 to C32: n-alcanes fractioned with GC chromatography .These are expressed as μg/g dwt of sediment.

UCM: Unresolved Complex mixture, LMW: low molecular weight; HMW: high molecular weight; ND: not detected

NAH varying from 60.7 to 1356.3 μg/g in the Eastern Harbor of Alexandria in Egypt (Aboul-Kassim et al., 1995). AH concentrations determined in Khniss sediments are relatively high, compared with those established in the sediments of the Gulf of Fos in France ranging from 2.5 μg/g to 80 μg/g (Mille et al., 2007) and in the sediment of Santos coastal area in Brazil which varied between 0.08 and 42.39 μg/g (Nishigima et al., 2001).

Aliphatic Biomarkers and Origins in Polluted Sites

Aliphatic hydrocarbons and NAH are considered as important petroleum fractions present in the marine sediments. Their sources are either natural, from photosynthesis by marine biota inhabiting the surface waters, or anthropogenic, from land run-off, fallout, and/or industrial input (Aboul-Kassim et al., 1995). The aliphatic hydrocarbon fraction is composed of n-alkanes, branched alkanes, isoprenoids and cyclic compounds. Their analysis can be used to fingerprint spilled oils and provides additional information on the source of hydrocarbon contamination and the extent of degradation of the oil spill (Tolosa et al., 2004). Petroleum compounds are generally readily identified by their gas chromatogram traces. NAH analysis was also carried out for aliphatic petroleum biomarkers identification (Table 4). Obtained GC traces show regular distribution ranged from n-C9 to n-C31 alkanes with equivalent distribution pattern of both odd carbon-numbered alkanes and even-carbon-numbered alkanes. In fact, GC trace analysis showed unimodal n-alkane distribution at KSA which is a distribution with single mode indicating unique source of pollution whereas bimodal distribution was found in KSC. In this last case a mixture of two normal distributions with the same variance but different means are observed in the GC traces which demonstrate the presence of two different sources of petroleum pollution (gasoline, crude oil, diesel…). It is recognized from the literature that both unimodal and bimodal n-alkane distributions ranging from n-C15 to n-C32 are characteristic of petroleum origin (Yang et al., 2009). Bimodal distribution is observed for certain oil products. It also may be due to the superposition of two different types of petroleum product sources (Yang et al., 2009). This possibility is more probable for the KSA sediments, considering the nature of the petroleum products input during the sampling period and the nature of maritime activities in this region. Thus, this distribution is the result of a mixed origin of two types of petroleum products which the first is a refined product such as kerosene, fuel reactor and diesel products. These products are characterized by the presence of the n-alkanes range between n-C6 and n-C26 with a maximum of n-C26. This is the case of the chromatogram obtained from the KSA sediment NAH. The absence of n-C6 may be due to the evaporation which is generally observed for low molecular weight hydrocarbons (LMW). The presence of n-C9 and n-C10 in the chromatograms may also indicate a recent contamination with light distillated product such as diesel or gasoline (Yang et al., 2009). Both gasoline and diesel are also characterized with central UCM (Unresolved Complex Mixture) similar to that present in KSA sediment GC trace. UCM is an indicator of petroleum contamination and n-alkanes biodegradation (Mille et al., 2007). The chromatograms obtained from KSC sediment are characterized by the presence of n-alkanes ranged from n-C6 to n-C26 with developed UCM below the n-C26. UCM values in khniss samples (Table 3) are relatively higher than those recorded in the Bay of Marseille (11-230 mg/Kg sed. Dry weight) (France) (Asia et al., 2009), but relatively low compared to those found in sediment from Sfax region-Tunisia (Zaghden et al., 2005). An equivalent distribution pattern of both odd carbon-numbered alkanes and even carbon-numbered alkanes resulting in a carbon preference index (CPI) near the unit was also found in KSA and KSC sediments (Table 4). CPI values are suggested to be a useful indicator of the relative contribution of n-alkanes from fossil hydrocarbons and biogenic emissions, and several studies have confirmed oil CPI values to be around 1.0 (Zheng et al., 2005, Wang et al., 2003; Kalaitzoglou et al., 2004). Table 4 also presents the different ratios of Pr/Ph, Pr/C17 and Ph/C18 which are used to identify the anthropogenic origin when ≤1. At the same ∑LMW/∑HMW (when ≥1) and UCM/∑n-alkanes (when >2), confirm petroleum origin in KSA and KSC (Table 4). C17/C29, NAR and TAR were however, suggesting the biogenic contribution in KSA and KSC (Table 4). The nC17/C29 ratio indicates the relative presence of allochthonous and autochthonous hydrocarbons in the samples; this confirms the limited contribution of marine origin at KSA and their dominance at KSC. In fact n-C29 is abundant in land plants and n-C17 is dominant in marine organisms. The terrigenous/aquatic ratio (TAR), which reflects the long-chain n-alkane (n-C27 + n-C29 + n- C31) to short-chain n-alkane (n-C15 + n-C17 + n-C19), can be useful to confirm the importance of terrigenous and aquatic inputs (Bourbonniere et al., 1996). Biogenic sources of

TABLE 4 ALIPHATIC AND AROMATIC BIOMARKERS IN KSA AND KSC SEDIMENTS

	Aliphatic Biomarkers								
	UCM/∑n-alcanes	CPI	Pr/Ph	Pr/C17	Ph/C18	C17/C29	NAR	TAR	∑LMW/∑HMW
KSA	3.93	1	1.08	2.74	0.31	0.03	0.008	6.37	0.23
KSC	6.75	1.2	1.08	2.74	0.31	1.59	0.26	1.27	1.07
	Aromatic biomarkers								
	ΣMphe/Phe	DMphe/Phe	Phe/Anth	Anth/Anth+Phe	Fluo/Pyr	Fluo/Fluo+Pyr			
KSA	3.78	0.59	nd	0	0	0			
KSC	8.62	0.15	2.61	0.28	0.04	0.07			

UCM/∑n-alcanes : Unresolved Complex Mixture/sum of n-alcanes ; CPI : Carbon Preference Index ; Pr : Pristane ; Ph : Phytane ; NAR : Natural n-Alkane Ratio; TAR : Terrigenous/Aquatic Ratio ; ∑LMW/∑HMW : sum of n-alcanes of low molecular weight / sum of n-alcanes of high molecular weight. DMPhe: 3,6-dimethylphenanthrene ; Fluo: Fluoranthene ;∑Mphe: 1-methylphenanthrene+2-methylphenanthrene ; Pyr: Pyrene ; Phe: Phenanthrene ; nd: non-determine ; Anth: Anthracene.

hydrocarbon come from both inputs. The TAR ratio was higher than 1 at KSA, but was near the unit in the KSC site (Table 4). The natural n-alkane ratio (NAR) is used to estimate the proportions of natural and petroleum n-alkanes (Mille et al., 2007). The values of NAR ratio is close to 0 for petroleum hydrocarbon and close to 1 for terrestrial or marine plants. In our study, no value close to 1 was registered at each of the investigated sites (Table 4). NAH analysis therefore shows that the limited biogenic source contribution is explained by the mix of terrestrial input and marine hydrocarbons. A fishing site is in fact located near several maritime activities. Observing the n-alkane distribution and performing a marker analysis enabled us to render a hypothesis of petroleum source contribution with limited biogenic contribution in both KSA and KSC sediments.

PAHs Distribution in Polluted Sediments

Aromatic hydrocarbons were analyzed to reveal the presence of PAHs contamination relative to pyrolytic or petrogenic origins in Khniss sediments. Table 5 presents the total PAHs (Σ17 PAHs) and individual PAH concentrations. Results show that Σ17PAHs concentrations vary from 6.95 ng/g in KSC to 14.59 ng/g in KSA. The Khniss coastal area is home to many small industries and maritime activities, which may explain the observable difference between the concentrations in the sediments. In fact this region is subject to hydrocarbon pollution by sewage, industrial and aquacultural waste, and other human activities. An understanding of the local and global extent and severity of marine environment contamination by fossil fuel hydrocarbons from various sources requires measuring the compounds of interest and comparing them in different regions. The total PAHs concentration we measured in the Khniss coastal region indicates that the sediment contamination is important, and comparable to that found in other locations around the world (Table 6). National comparison revealed, elevated PAH concentrations in the lagoon of Bizerte (83.3 ng/g to 447) (Trabelsi et al., 2005) and in the coastal area of Sfax and Kerkennah (113 ng/g to 10720 ng/g) (Zaghden et al., 2007). This comparison also showed that the Khniss region showed lower sedimentary concentrations of PAHs compared with those found in the sediment of Vendres harbor in France (145 to 6940 ng/g (Baumard et al., 1998), and Haihe River in Chine (775 to 255372 ng/g) (Bin et al., 2007). The distribution of the individual PAH in KSA and KSC is reported in Table 5. In sediment from KSA

TABLE 5 PAH LEVELS (ng/g DRY WEIGHT) IN KSA AND KSC SEDIMENTS

	KSA ng/g	KSC ng/g
Naph	$3.47\ 10^{-1}$	$1.14\ 10^{-1}$
1menaph	-	$2.10\ 10^{-1}$
1enaph	-	$1.98\ 10^{-1}$
Ac	-	$2.23\ 10^{-1}$
Ace	1.62	$1.82\ 10^{-1}$
2,3,6 trimenaph	-	$3.86\ 10^{-2}$
Flu	$5.03\ 10^{-2}$	$7.19\ 10^{-3}$
Phe	$4.31\ 10^{-3}$	$8.70\ 10^{-2}$
Anth	-	$3.34\ 10^{-2}$
2-mephe	$1.63\ 10^{-2}$	$8.54\ 10^{-2}$
1-mephe	-	$6.65\ 10^{-1}$
3,6-dimephe	$2.54\ 10^{-3}$	$1.28\ 10^{-2}$
Fluo	-	$7.55\ 10^{-2}$
Pyr	$1.80\ 10^{-3}$	1.93
1-mepyr	$3.29\ 10^{-3}$	$5.16\ 10^{-1}$
Chr	12.35	1.62
Pery	$1.83\ 10^{-1}$	$9.52\ 10^{-1}$
∑ PAH	14.59	6.95
∑ 2-3 rings	2.05	1.86
∑ 4-5 rings	12.5	5.1

Abbreviations: Naphthalene (Naph), 1methyl-naphthalene (1menaph), 1ethyl-naphthalene (1enaph), acenaphthylene (Ac), acenaphthene (Ace), 2,3,6 trimethyl-naphthalene (2,3,6 trimenaph), fluorene (Flu), phenanthrene (Phe), 2-methyl-phenanthrene (2-mephe), 1-methyl phenanthrene (1-mephe), 3.6-dimethyl-phenanthrene (3,6-dimephe), fluoranthene (Fluo), pyrene (Pyr), 1-methyl-pyrene (1-mepyr), anthracene (Anth), chrysene (Chr), and perylene (Pery): these are expressed as ng/g of sediment by using GC analyses.

TABLE 6 COMPARISON OF SEDIMENT PAH CONCENTRATION (ng/g DRY WEIGHT) OF KHENISS COASTAL WITH THOSE IN OTHERS REGIONS

Sediments	PAHs (ng/g)	Refernces
Khniss coast, Tunisia	6.95-14.59	This study
Jarzouna coast, Tunisia	916.4-4541.1	Zrafi-Nouira et al 2008
Lagune of Bizerte, Tunisia	83.3-447	Trabelsi et al 2005
Kerkennah-Sfax coast, Tunisia	113-10720	Zaghden et al 2005
Vendres harbour, France	145-6940	Baumard et al 1998
Baltic Sea	720-1900	Witt et al 1995
Porto Torres harbour, Sardaigne, Italie	70-1210	De luca et al 2004
Baie Kyeonggi, Corée	9.1-1400	Kim et al 1999
Malaisie	4-924	Zakaria et al 2002
Haihe River, Chine	775-255372	Bin et al 2007
Deep Baie, Chine	353.8	Qiu et al 2009

site, we noticed the absence of 1-methyl-Naphthalene, 1-ethyl-Naphthalene, Acenaphthene, 2,3,6-trimethyl-Naphthalene, Anthracene, 1-methyl phenanthrene and Fluoranthene and the relative abundance of Naphthalene, Acenaphthene, Fluorene, Chrysene and Perylene. In KSC sediment; results show the presence of all target PAHs with the abundance of Fluorene, Pyrene, 1-methyl Pyrene, Chrysene and Perylene. Chrysene is the most important representative toxic PAH, in KSC (Table 5). High concentrations of chrysene can be associated with the weathering effect in crude oil's chemical composition change. In addition, high concentrations of Chrysene which is considered as a "conservative" PAH due to its resistance to weathering and bacterial degradation (Wang et al., 2003); indicates petroleum contamination input in this site. Higher concentrations of perylene could however, result from terrigenous precursors whose diagenetic degradation could lead to the formation of perylene and indicates biogenic contribution (Venkatesan et al., 1988).

PAH Compound Profiles and Origins

The analysis of the Low Molecular Weight PAHs (LMW-PAHs) and High Molecular Weight PAHs (HMW-PAHs) were useful in the characterization of petroleum pollution and an interpretational advantage in fingerprinting sources of spilled oils and for providing additional diagnostic information. Analysis of individual PAH characteristics from Khniss sediments showed the predominance of HMW-PAH (4-5-ring PAH). Petrogenic source contains relatively higher concentrations of individual LMW-PAH (2-3-ring PAH) compounds (Tolosa et al., 1996), while a high-molecular weight parent PAHs dominance is a typical characteristic of a pyrolytic source: combustion origin (Budzinski et al., 1997). HMW-PAHs have been recognized as directly carcinogenic and evidence suggests that the environmental persistence and genotoxicity of PAHs increase as the molecular size of the PAHs increases up to four or five fused benzene rings (Cerniglia et al., 1992). On the other hand dominance of the HMW-PAHs can be due to their strongly adsorbed by sediments whereas low molecular weight PAHs are subject to several degradation (Wang et al., 2003; Jones et al., 1986). Pyrolytic (fossil fuel combustion and vegetation fires) and petrogenic (oil spill and petroleum products inputs) are the primary sources of PAHs found in coastal marine sediments. Our results are comparable to those showing predominance of high molecular weight in the surface sediment of Jiaozhou Bay in China (Wang et al., 2006). The identification of the pollution origin is also based on the identification of compounds with specific sources. In fact, the presence of Fluoranthene (Fluo) and Pyrene (Pyr) indicates the importance of pyrolytic inputs since these compounds are considered as products formed from the condensation of aromatic compounds of low molecular weight at high temperature (Zeng et al., 1997; Wang et al., 1999). Chrysene is considered as a preserved biomarker of PAHs and it was selected to be a good marker of petroleum compounds because of its resistance to abiotic factors and bacterial degradation (Wang et al., 2003). Chrysene showed relatively high concentration in KSA site sediment, reflecting a petrogenic contamination. In addition, some diagnostic ratios (Fluo/Pyr, Phe/Anth, Fluo/Fluo+Pyr, Anth/Anth and ΣMphe+Phe/Phe) were used to distinguish between petrogenic and pyrolytic PAHs (Bin et al., 2007) (Table 4). Phe/Anth ratio under 3 indicates pyrolytic origin and it indicates petrogenic origin when it is over 3 (Ke et al., 2002; Tolosa et al., 2009).

On the other hand, a ratio (Anth/Anth+Phe) <0.1 indicates a petrogenic source, while this ratio indicates a combustion source when it is greater than 0.1. The ratio of Fluo/Pyr <1 is characteristic of a petrogenic source and the ratio Fluo/Pyr >1 characterizes a pyrolytic source. The ratio (Fluo /Pyr + Fluo) differentiates between petroleum, wood, coal and

plants combustion. When Fluo/Pyr+Fluo <0.5, it is generally associated with petrogenic source as a characteristic of fuel combustion (gasoline, diesel and crude oil), while when this ratio exceeds 0.5 it characterized pyrolytic sources (kerosene, wood, terrestrial plants and coal combustion) (Yunker et al., 2002; Mille et al., 2007). Phe/Anth and Anth/Anth+Phe ratios indicate that PAHs present in KSC sediment derived from pyrolytic sources. This pyrolytic origin is however not exclusive since the values of Fluo/Pyr and Fluo/Pyr+Fluo ratios show the contribution of oil sources (lubricating oils) in KSA and KSC sediment indicating mixed origin of PAHs.

Conclusion

This work represents the first detailed study on the distribution of hydrocarbons in the Khniss Tunisian-Coast sediments, their characterization and their possible sources. It extends our understanding of the current TH, NAH, AH and PAHs contamination status in this Mediterranean area. Hydrocarbons concentrations are relatively high compared to sedimentary concentrations along the Mediterranean coasts. Analysis of aliphatic, aromatic and PAHs hydrocarbons suggests an anthropogenic contamination in KSA and KSC sites, while hydrocarbons in KSB and KSD sites were characterized by biogenic sources. Hydrocarbon sources may therefore be related to wastewater and wadi Khniss discharges, harbor activities, fishing industries, atmospheric emission and extensive maritime traffic.

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